DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: ~~11/22/02~~ 12/03/02

From: Gloria Chang, IDS/Pharmacist, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

New Dietary Ingredient: BioDiamed (Mormadica charantia L.)

Firm: Kelatron Corporation World Headquarters

Date Received by FDA: 2/11/02

90-Day Date: 5/12/02

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Gloria Chang

Gloria Chang, IDS/Pharmacist

Attachments

95S-0316

RPT 114

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD

APR 26 2002

Mary Ann Coral-Amasifuen
Kelatron Corporation World Headquarters
1675 West 2750 South
Ogden, Utah 84401

Dear Ms. Coral-Amasifuen:

This is in response to four separate notifications you submitted pursuant to 21 U.S.C. 350b(a)(2). All four notifications were received by the Food and Drug Administration (FDA) on January 3, 2002, followed by an addendum dated January 10, 2002. In follow up, we contacted you by telephone on January 14, 2002 notifying you that the notifications were incomplete (see background of follow up below). Subsequently, you sent addendums dated January 18, and February 5, 2002. We received your last addendum for your notifications dated February 5, 2002 on February 11, 2002. Therefore, the effective filing date for all four notifications is February 11, 2002.

As noted above, we contacted you by telephone on January 14, 2002 notifying you that the notifications were incomplete in that they did not contain levels of the dietary ingredients, conditions of use, or copies of the full-text journal articles corresponding to the abstracts you sent us. We explained that the requested information would have to be submitted in triplicate (3 copies) if we were to consider these references in our review. On January 24, 2002, we called you again and left a message that the addendums that you sent dated January 18, 2002, did not contain the levels of the new dietary ingredients as requested.

Each notification concerned a different botanical that you assert is a new dietary ingredient. The botanicals are listed below by the Latin binomial name, plant form, and product name as stated in your notifications.

Vitex negundo L. (pure leaf powder) -- BioVitaflu/BioVitabronch
Blumea balsamifera L. (pure leaf power) -- BioRenal
Mormadica charantia L.- Makiling v. (pure leaf powder) -- BioDiamed
Lagerstroemia specious L. (pure leaf powder) -- BioDiamend

The law at 21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor submit certain information to FDA at least 75 days before a new dietary ingredient or a dietary supplement containing it is introduced or delivered for introduction into interstate commerce. This information must include the basis on which the manufacturer or distributor has concluded that the new dietary ingredient or a dietary supplement containing it will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C. 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the product's labeling, will reasonably be expected

to be safe. If this requirement is not met, the new dietary ingredient or dietary supplement containing it is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B), because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has considered the information in your notification and has several significant concerns. Based on the information in your notification for all four botanical ingredients, FDA has determined that the information submitted suggests that the intended uses imply or represent treatment of disease. The following are examples.

- The botanical ingredient *Vitex negundo* L., the product name "BioVitaflu/BioVitabronch" implies a recognizable disease condition, the "flu". FDA considers a brand name that includes a disease name or a clearly recognizable derivation of a disease name to be a disease claim. (See 21 CFR 101.93(g)(2)(iv)(A).)
- Under the conditions of use for the botanical ingredient *Blumea balsamifera* L. (BioRenal) you state that BioRenal might be effective as a diuretic and as an anti-urolithiasis agent (chronic formation of kidney stones).
- Under the conditions of use for the botanical ingredient *Mormadica charantia* L.-Makiling v. (BioDiamed) you state that the recommended use is that it may be helpful for blood sugar regulation and type II diabetes mellitus.
- Under the conditions of use for the botanical ingredient *Lagerstroemia specious* L.-(BioDiamend) you state that clinical trials indicated that BioDiamend may have some blood sugar lowering properties in vivo and therefore the recommended use is that it may be helpful for blood sugar regulation and type II diabetes mellitus.

Please be advised that any representation that a product is intended for the diagnosis, cure, mitigation, treatment or prevention of disease in man or animals suggests that it is a drug, as defined in 21 U.S.C. § 321(g)(1)(B), and would be subject to regulation under the drug provisions of the Federal Food, Drug and Cosmetic Act. All drugs must be approved by FDA before they can be marketed in the United States. If you wish to market your products as drugs, you should contact FDA's Center for Drug Evaluation and Research (CDER), Office of Compliance, HFD-310, 7520 Standish Place, Rockville, Maryland 20855.

FDA also has concerns about the evidence on which you rely to support your conclusion that the four botanical ingredients in your notifications will be reasonably expected to be safe for the suggested or intended uses.

Much of the history of use information you submitted appears to be selected pages printed from commercial magazines or promotional literature. Some of the sources of these articles were not identified nor were the specific ingredients in your notifications mentioned in the articles. These articles primarily focus on anecdotal use for disease conditions and do not address safety. The statements in these articles cannot be validated and are not corroborated

by scientific data. Although requested, you did not provide us with photostatic copies or reprints of all of the abstracts or the complete reference citation for what appears to be an excerpt from a reference book. Consequently, those abstracts and excerpts were not considered in our review.

We are also unsure if the botanical ingredients described in some of the scientific literature were the same as those described in your notifications. Further, we are not sure if the specific genus, species, and author citations are correct for two of the botanical ingredients. Although we searched a number of botanical databases, we could not find the specific Latin binomial names *Mormadica charantia* L. and *Lagerstroemia specious* L as stated in your notifications. We are aware of the Latin binomial names *Momordica charantia* L. or *Momordica charantia* Linn. and *Lagerstroemia speciosa* L. or *Lagerstroemia speciosa* (L.) Pers. However, when referring to your botanical ingredients in this letter, we will be using the Latin binomial names as stated in your notifications.

We also have concerns regarding the scientific information that was submitted. Most of the scientific articles and unpublished reports in your notifications primarily address use of the study ingredients as drugs to treat specific disease conditions and do not provide adequate evidence of the safe use of the specific ingredient. Also, it was not clear if the ingredients used in some of the studies were the same ingredients (genus, species, and author citation), the same part of the plant, or the levels per serving dose, as those stated in your notifications.

In your notification on *Vitex negundo* L (BioFlu/Bio Vitabronch), you submitted a summary of an unpublished, uncontrolled, open label study evaluating the safety and efficacy of *Vitex negundo* L (Lagundi) tablets as an antitussive agent. The trial titled Section 5.2:Phase II Clinical Trial was conducted from January to December 1984. Twenty-five subjects were enrolled, 20 children and 5 adults. Subjects were described as having acute asthma (n=4) or upper-respiratory, non-bacterial infection (n=21). There was a single concluding statement of safety that noted that there were no untoward side effects noted or volunteered. No details or specific data on safety was provided. We also note that the actual dose level in each tablet was not stated. Further, subjects with present or past disease conditions were explicitly not enrolled in the trial as stated in the exclusion criteria of the study. This is of particular concern since under your conditions of use there are no recommendations to restrict its use in persons with pre-existing disease conditions.

In the report of a randomized study comparing lagundi (15 mg/kg taken every 8 hours for 3 days) to theophylline (3 mg/kg taken every 8 hours for 3 days) for the treatment of acute asthmatic exacerbation (a disease condition), forty-three subjects were enrolled, however; 3 subjects dropped out after 24 hours. Twenty of the subjects were exposed to lagundi. The analysis was done on forty subjects, 6 males and 34 females. For almost all outcome measures the theophylline group was superior to the lagundi group. Adverse events were noted for 8 theophylline subjects and 5 in the lagundi group. In the lagundi group, the side effects noted were emesis (2 cases), palmar desquamation (2 cases) and increased urinary frequency (1 case). The author did not comment on the subjects that developed palmar

desquamation. The author also expressed concerns about the inadequacy of this study and recommended further evaluation and investigation of lagundi.

We also have concerns regarding the short exposure time to lagundi. The total clinical exposure cited as a safety database consists of only approximately 45 individuals with only a maximum exposure to lagundi of 72 hours. Considering that you did not indicate any limitation or duration of use, these studies do not address chronic use or long term use. Further, we have concerns that subpopulations with present or past medical conditions that were excluded in the study, were not recommended for exclusion under your conditions of use. Accordingly, the study cannot support the conclusion that lagundi is reasonably expected to be safe if marketed as a new dietary ingredient for the intended or suggested use.

In the notification for *Blumea balsamifer* L. (BioRenal), you submitted sections of a larger unpublished study labeled as "7.0 CLINICAL TRIALS." The subsections are; 7.1 "Phase I: Sambong Tablet as Diuretic", 7.2 "Phase II:Clinical Trial of Sambong Tablet as Diuretic," 7.3 "Phase II:Sambong Tablet as anti-urolithiasis," 7.4, "Phase III clinical Trial of *Blumea balsamifer* L (Sambong) tablet in the treatment of urinary tract stone: a randomized double-blind placebo-controlled study", and 7.5 "Extended Phase III Open Trial of *Blumea balsamifer* L (Sambong) for the treatment of urinary tract stones."

All of the studies were small. Overall, 59 subjects were exposed to Sambong across all 5 studies. Exposure time ranged from 2 days to a maximum of approximately 6 weeks. Most of the exposures were less than 6 weeks.

In the studies for diuretic use, we have the following specific comments. No mechanism for the diuretic activity was ascertained, yet based on the conclusions reached that the diuretic effect of Sambong was comparable to thiazide diuretics, Sambong use may pose a safety risk in a normal population or in a subpopulation who may be also using other diuretics. The studies did not sufficiently address safety. Based on the conclusions in the study that Sambong tablets produced statistically significant diuresis and chloriuresis comparable to hydrochlorthiazide given at 50 mg in 2 divided doses, we have concerns that this may pose an electrolyte imbalance risk in normal populations or in a subpopulation with certain present or past medical conditions. Your recommended conditions of use only excluded use in lactating or pregnant women. Your recommended use in adults 18 years old and over neither included instructions on limitations or duration of use nor excluded use for any other populations that may be at risk either for using diuretics or due to concurrent use of other diuretic agents.

In addition, we have concerns regarding the implied use of BioRenal to treat or prevent kidney stones, a disease condition. We have significant safety concerns that consumers will not be able to self diagnose this specific disease condition and that prolonging medical treatment may lead to more serious health consequences.

In your notification for *Mormadica charantia* L.- Makiling v. (BioDiamed), the only full text journal article, was a general summary on the anti-diabetic properties and phytochemistry of a

botanical *Momordica charantia* L. Please note the difference in the Latin binomial names for your botanical ingredient and the botanical cited in the article. The article primarily focuses on general efficacy, and not the safety of the seeds or juice of the plant. It does not address the specific plant part or form (the pure leaf powder) or the serving levels as that of your ingredient. Further, the *in vivo* animal studies information presented a general overview of referenced toxicity studies and focused primarily on the juice or extracts of Karela. You did not provide the referenced full text journal articles in your notification. We are unsure if Karela is the same plant source or plant form as your ingredient. Nonetheless, the animal toxicity information did not provide any dosing levels used nor did it address the specific plant form described in your notification.

Thus, we conclude that the evidence of safety from the article was minimal or lacking and no conclusions of safety can be drawn from the report. We also cannot draw any safety conclusions from the other published report on the hyperglycemic activity of polypeptides of a plant source (fruit, seeds, and tissue). That report focuses on a peptide isolated from the seeds and tissue of a botanical variety, *Momordica charantia* Linn. and does not describe the specific plant part (pure dried leaf powder) described in your notification. Further, the report primarily addresses hypoglycemic activity of the peptide and the only safety information is a statement that referenced a study using a polypeptide-p-ZnCl in three juvenile patients. A photostatic copy or reprint of the full published text of that citation reference was not included in your submission. Thus, no conclusions regarding safety can be drawn from the report.

In your notification for *Lagerstroemia specious* L., the study submitted appears to be an unpublished trial titled "The Clinical Study on the Water Extract of Leaves of *Lagerstroemia specious* L for Mild Cases of Diabetes Mellitus." Twenty-four subjects over the age of 20 years were studied. There is very little information on safety in this report and it is unclear if the study was a single or double-blinded study, a critical concern in safety analysis. The only statement regarding safety was a statement that all 24 subjects did not have any adverse effects. In the absence of detailed safety data and the small size of the study, there is very little evidence to conclude that the ingredient can be reasonably expected to be safe for its intended or suggested use.

Overall, the evidence of safety provided for all four of the dietary ingredients submitted is either minimal or lacking. All of the supporting studies were of a short duration, without any evidence demonstrating safety with chronic exposure. You indicated that under conditions of use these ingredients in general, were to be recommended for use in adults (18 and over) and were not to be used by lactating or pregnant women. However, the study exclusion criteria specifically excluded subpopulations with certain medical conditions from the studies. This may be of particular concern, because under your conditions of use you did not indicate any limit or duration of use for the four botanicals and persons excluded from clinical trials are not excluded under your recommended conditions of use.

We have determined that the history of use information you submitted in all four of your notifications has limited utility in evaluating the safety of these ingredients if marketed as

dietary supplements. In conclusion, the information in your notifications does not provide an adequate basis to conclude that *Vitex negundo* L., *Blumea balsamifera* L., *Mormadica charantia* L.- Makiling v., and *Lagerstroemia specious* L. are reasonably expected to be safe when used under the recommended or suggested conditions of use. Therefore, any product containing any of the botanicals listed in your notifications as *Vitex negundo* L., *Blumea balsamifera* L., *Mormadica charantia* L.- Makiling v., and *Lagerstroemia specious* L. may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains one or more new dietary ingredients at levels for which there is inadequate information to provide reasonable assurance that they will not present a significant or unreasonable risk of illness or injury. Adulterated or unsafe dietary supplements are prohibited under 21 U.S.C. 331(a) and (v) from being introduced or delivered for introduction into interstate commerce.

Your notifications will be kept confidential for 90 days after the filing date of February 11, 2002. After May 11, 2002, the four notifications will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. However, any trade secret or otherwise confidential commercial information in the notifications will not be disclosed to the public.

Prior to May 11, 2002, you may wish to identify in writing specifically what information in your notifications you believe is proprietary for FDA's consideration. Nevertheless, our Center's Freedom of Information Officer has the authority to make the final decision about what information in the notifications should be redacted before they are posted at Dockets.

If you have any questions concerning this matter, please contact us at (301) 436-2371.

Sincerely yours,

Felicia B. Satchell

Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

KELATRON CORPORATION
1675 West 2750 South • Ogden, Utah 84401
Phone: 801-394-4558 • Fax: 801-394-4559
Corporate Sales Office Phone: 801-627-3050 • Fax: 801-612-9191
Toll Free: 1-800-201-6896
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Mr. Gary Coody
Office of Nutritional Products
Labeling and Dietary Supplements (HFS-805)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Md. 20740

Dear Mr. Coody,

In reference to the submission of information on the botanicals trademarked **Biodiamed, Biodiamend Biorenal and Biovitabronch/Biovitaflu** in accordance with the regulation:
TITLE: 21 Food And Drugs
Chapter I – Food and Drug Administration
Dept of Health and Human Services
Part 190 – Dietary Supplements
Subpart B—New Dietary ingredient Notification
Sec. 190.6 Requirement for premarket notification

Please accept the enclosed modified pages which include *Directions* (for use) under the *Condition of use* clause.
Also enclosed are additional materials (clinical trial data)on Biorenal for your review.
I believe this was the missing information.

Please call me directly at my office in North Carolina, 252-234-7160 if further information is needed.

Thank you,

Mary Ann Coral-Amasifuen

From:
Mary Ann Coral-Amasifuen
Kelatron Corporation World Headquarters
1675 West 2750 South
Ogden, Utah 8440 Phone (801) 394-4558
Kelatron Corporation Botanical Division
2145 Barefoot Park, SW
Wilson, North Carolina 27893 Phone: (252) 234-7160

To:
Office of Nutritional Products
Labeling and Dietary Supplements (HFS-805)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Md. 20740
Atten: Gary Coody

In accordance with:
TITLE: 21 Food And Drugs
Chapter I – Food and Drug Administration
Dept of Health and Human Services
Part 190 – Dietary Supplements
Subpart B—New Dietary ingredient Notification
Sec. 190.6 Requirement for premarket notification

(1) **Name and address of distributor**: Kelatron Corporation
1675 West 2750 South
Ogden, Utah 84401

(2) **Name of new dietary ingredient**: BioDiamed (*Mormadica charantia*,L – Makiling v.)

(3) **Description of new ingredient:** Biodiamed is the bulk pure leaf powder of the plant *Momordica charantia*, L. makiling variety harvested for medicinal purposes in the Philippines. There has been clinical research done on the effectiveness of this plant for lowering blood sugar. It is currently in use in the Asian market under the name Ampayala which is the local name for the plant in southeast Asia.

(3) (i) **Level of new ingredient**: the product contains only the pure plant leaf powder and no other substance to be sold in bulk powder form to retail manufacturers.

(3) (ii) **Condition of use**: In general, to be used by adults (18 and over). Not to be used by lactating or pregnant women. Directions for use: One to three 500 mg capsules three times per day.

(4) **History of use**: *see attachment 4A*

(5) **Signature** _____ **Date** 2.5.02

From:
Mary Ann Coral-Amasifuen
Kelatron Corporation World Headquarters
1675 West 2750 South
Ogden, Utah 8440 Phone (801) 394-4558
Kelatron Corporation Botanical Division
2145 Barefoot Park, SW
Wilson, North Carolina 27893 Phone: (252) 234-7160

To:
Office of Nutritional Products
Labeling and Dietary Supplements (HFS-805)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Md. 20740
Atten: Gary Coody

In accordance with:
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Chapter I – Food and Drug Administration
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(3) (ii) **Condition of use**: In general, to be used by adults (18 and over). Not to be used by lactating or pregnant women.

(4) **History of use**: *see attachment 4A*

(5) **Signature** _____ **Date** 1-18-02

January 18, 2002

MR. Coody,

I have made corrections to the Conditions of use portion in three of the applications.

We will send the full text article and remaining Condition of use modification when I arrive back to my office in North Carolina.

It would be helpful if you would log in the botanical products that are in compliance with the information requested.

Thank you,

Mary Ann Coat-Emerson

From:
Mary Ann Coral-Amasifuen
Kelatron Corporation World Headquarters
1675 West 2750 South
Ogden, Utah 84401 Phone (801) 394-4558
Kelatron Corporation Botanical Division
2145 Barefoot Park, SW
Wilson, North Carolina 27893 Phone: (252) 234-7160

To:
Office of Nutritional Products
Labeling and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

In accordance with:
TITLE: 21 Food And Drugs
Chapter I – Food and Drug Administration
Dept of Health and Human Services
Part 190 – Dietary Supplements
Subpart B—New Dietary ingredient Notification
Sec. 190.6 Requirement for premarket notification

(1) **Name and address of distributor**: Kelatron Corporation
1675 West 2750 South
Ogden, Utah 84401

(2) **Name of new dietary ingredient**: BioDiamed (*Mormadica charantia*,L – Makiling v.)

(3) **Description of new ingredient:** Biodiamed is the bulk pure leaf powder of the plant *Momordica charantia*, L. makiling variety harvested for medicinal purposes in the Philippines. There has been clinical research done on the effectiveness of this plant for lowering blood sugar. It is currently in use in the Asian market under the name Ampayala which is the local name for the plant in southeast Asia.

(3) (i) **Level of new ingredient**: the product contains only the pure plant leaf powder and no other substance to be sold in bulk powder form to retail manufacturers.

(3) (ii) **Condition of use**: clinical trials indicated that BioDiamed may have some blood sugar lowering properties in vivo and therefore the recommended use is that it may be helpful for blood sugar regulation and type II Diabetes mellitus.

(4) **History of use**: *see attachment 4A*

(5) **Signature** [signature] **Date** 12-10-01

RECEIVED 1/3/02

ATTACH: 4A

Ampalaya

Each capsule contains 500mg Ampalaya leaves (***Momordica Charantia*** L.,Makiling variety).

OTHER NAMES: *Ampalaya* (Tag.) *Amargoso* (Bik.) *Palia* (Bis., Bon.,If.) *Paria* (Bik, Ilk,Sul.) *Bitter gourd* (Engl.)

Ampalaya is a very common and highly nutritious vegetable widely cultivated throughout the Philippines. Both the leaves and the fruits are edible and rich in Vitamins A, B and C. It is also a good source of Iron, Calcium and Phosphorus. According to the FNRI, each 100gm of cooked ampalaya leaves contain the following nutrients:

Beta Carotene (Vitamin A)	3085 ug
Thiamine (Vitamin B1)	0.07 mg
Riboflavin (Vitamin B2)	0.23
Niacin	1.3 mg
Ascorbic Acid (Vitamin C)	41 mg
Iron	0.6 mg
Phosphorus	51 mg
Calcium	151 mg
Calories	39 kcal

Our ampalaya vines are cultivated and nurtured in an exclusively organic and environment-friendly farm. The leaves are harvested only by trained personnel and processed into capsules in a BFAD-registered Pharmaceutical facility complying with Current Good Manufacturing Practice (CGMP) standards.

Traditional / Folkloric / Ethnomedical Uses

Traditional healers have long used the leaves, roots and fruits for fever, intestinal parasitism, cough, diarrhea, burns, skin diseases and headaches. It is also commonly used for diabetes and as a blood tonic.

The National Integrated Research Program on Medicinal Plants (NIRPROMP) has confirmed the safety and efficacy of Ampalaya (Makiling variety) in lowering blood sugar levels among diabetic patients. Presently, the Department of Health is promoting Ampalaya (Makiling variety) as one of the ten (10) plants for public consumption. **Our Ampalaya capsules contain only the Makiling variety which has been studied by the NIRPROMP and validated for its blood sugar lowering effect.**

RECOMMENDED DOSE : Initially 2 to 3 capsules 3x daily. Dose may be adjusted depending on blood sugar levels.

1 : *Bangladesh Med Res Counc Bull* 1999 Apr;25(1):11-3 Related Articles, Books, LinkOut

Effect of Momordica charantia (Karolla) extracts on fasting and postprandial serum glucose levels in NIDDM patients.

Ahmad N, Hassan MR, Halder H, Bennoor KS

Department of Pathology, Sher-e-Bangla Medical College, Barisal.

Effect of Momordica charantia, a bitter vegetable popularly known as Karolla, on fasting and post prandial (2 hours after 75 gm oral glucose intake) serum glucose levels were studied in 100 cases of moderate non-insulin dependent diabetic subjects. Drinking of the aqueous homogenized suspension of the vegetable pulp led to significant reduction ($p < 0.001$) of both fasting and post-prandial serum glucose levels. This hypoglycaemic action was observed in 86 (86%) cases. Five cases (5%) showed lowering of fasting serum glucose only.

Publication Types:
- Clinical trial
- Controlled clinical trial

PMID: 10758656, UI: 20221826

Rivera [488] conducted a preliminary chemical investigation of the drug, *Momordica charantia* L., which is used empirically in Puerto Rico for the treatment of diabetes mellitus. His findings are as follows:

1. Determinations of total solids, ether-soluble extract, alcohol-soluble extract, crude protein, crude fiber, carbohydrates, ash, calcium, and phosphorus in the roots, stems, leaves and green fruit of the plant, as well as in the whole plant itself.
2. Data on the amounts of extractive removed by various solvents and the influence of particle size on the amount of extractive.
3. Results of the application of Dragendorff's method of successive extractions of the drug with petroleum ether, ethyl ether, alcohol, and water.

These data, when reviewed, indicate:

a. That the petroleum ether extractive includes a highly aromatic ethereal oil, a fixed oil, traces of free fatty acids and carotene;
b. That the ethyl ether portion contains chlorophyll, a glucoside-like substance and resin;
c. That the alcohol fraction contains alkaloids (probably two), one of which is Momordicin, a glucoside-like substance, and a crystalline substance of unknown nature which is being investigated further;
d. That the water-soluble extractive includes a saponin-like substance and mucilaginous bodies.

The roots and the leaves are official in the Mexican (1–4) Pharmacopœia.[92]

Steyn [561] quotes Descourtilz, who states that two or three drachms of the fruit taken internally will kill a dog. Rivera [488] says that the drug exerts at least some hypoglycemic action on rabbits.

In the Philippines it is reported that the juice expressed from the green fruit is given for chronic colitis. It is also found to be good for bacillary dysentery. It probably acts as an astringent. The dose is ½ glassful every other day. The juice of the leaves is given in the amount of a teaspoonful daily for children's coughs. Father de Sta. Maria [505] mentions the early use of the leaves and shoot as a vulnerary. Guerrero [249] reports that the sap of the leaves is used as a parasiticide, and the fruit, when macerated in oil, as a vulnerary.

According to Greshoff [234] the vine is used in Batavia as an anthelmintic, purgative, and emetic. Rivera [488] reports that

[92] Bruntz and Jaloux, p. 209.
[234] Greshoff, p. 88.
[249] Guerrero, p. 242.
[488] Rivera, p. 295.
[505] De Sta. Maria, p. 26.
[561] Steyn, p. 389.

in Cuba the plant is used in the treatment of diabetes mellitus. It is also used for wounds which are refractive to other kinds of treatment, for skin diseases, and for sterility in women. Rivera [488] quotes Diaz, who mentions the use of the plant in Puerto Rico in the treatment of diabetes. The vine is well-known as a drug for the treatment of chronic ulcers in the stomach.

Dalziel [155] reports that the root is sometimes used as an ingredient in aphrodisiac prescriptions and, along with the fruit or seeds, is also used as an abortifacient, as well as a remedy for urethral discharges. Waddell [600] says that a decoction of the root causes abortion. Watt [603] states that in India the root is used as an astringent, and is applied externally to haemorrhoids.

According to Holland [284] a decoction of the leaves is used as a stomachic in Lagos. Dymock [179] reports that the leaves are administered as an anthelmintic and are applied externally in leprosy. Menaut [367] considers the leaves as antipyretic. Drury [172] quotes Rheede, who states that the whole plant, pulverized, is a good specific externally applied in leprosy and malignant ulcers. Burkill [94] reports that it is common to pound the leaves and apply them to skin diseases in India, Malaya, and elsewhere. Burkill and Haniff [93] state that they are applied in cases of burns and scalds, and diarrhœa. Gimlette and Burkill [220] report that the leaves are applied as a poultice for headaches. Watt [603] says that an infusion of the leaves acts as a febrifuge. Kirtikar and Basu [316] report that the juice of the fresh leaves acts as a mild purgative for children. Ainslie [6] reports that in Jamaica the natives use boiled leaves and a decoction of the plant itself equally often to promote the lochiae. De Grosourdy [240] writes that in the Antilles, a sweetened decoction of the leaves is considered a powerful emmenagogue, and an effective vermifuge.

Gimlette and Burkill [220] state that the flowers enter into an infusion for asthma.

[6] Ainslie, p. 274.
[93] Burkill and Haniff, p. 205.
[94] Burkill, p. 1485.
[155] Dalziel, p. 62.
[172] Drury, p. 295.
[179] Dymock, p. 340.
[220] Gimlette and Burkill, pp. 333, 358, 360.
[240] De Grosourdy, v. 3, p. 215.
[284] Holland, p. 333.
[316] Kirtikar and Basu, p. 590.
[367] Menaut, p. 239.
[488] Rivera, p. 295.
[600] Waddell, pp. 296, 540.
[603] Watt, v. 5, p. 256.

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OTHER NAMES : *Ampalaya* (Tag.) *Amargoso* (Bik.) *Palia* (Bis., Bon., If.) *Paria* (Bik, Ilk, Sul.) *Bitter gourd* (Engl.)

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Review article

Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (Cucurbitaceae)

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Contents

Summary

Unripe fruit, seeds and aerial parts of *Momordica charantia* Linn. (Cucurbitaceae) have been used in various parts of the world to treat diabetes. Oral administration of the fruit juice or seed powder causes a reduction in fasting blood glucose and improves glucose tolerance in normal and diabetic animals and in humans. Animal and *in vitro* data support both insulin secretagogue and insulinomimetic activity of the fruit. However, enhanced insulin levels *in vivo* in response to its administration have not been observed. Although a wide range of compounds have been isolated from *Momordica charantia*, notably steroidal compounds and proteins, the orally active antidiabetic principle has not been adequately identified. A polypeptide, p-insulin, produces hypoglycaemic effects in humans and animals on subcutaneous injection, but oral activity is questionable. Other reported hypoglycaemic principles from *Momordica charantia* include the sterol glucoside mixture charantin (fruit) and the pyrimidine nucleoside vicine (seeds). However these are only effective at doses too high to account for all the activity of the plant extract. Principal toxicity of *Momordica charantia* in animals is to the liver and reproductive system. These effects have not been reported in humans despite widespread use of the fruit medicinally and as a vegetable.

Keywords: *Momordica charantia*; Cucurbitaceae; anti-diabetic; charantin; p-insulin; phytochemistry.

Introduction

Many plants have been used for the treatment of diabetes mellitus in traditional systems of medicine throughout the world. Indeed, along with dietary measures, plant preparations formed the basis of the treatment of the disease until the introduction of insulin in 1922. A number of review articles have been published on the traditional use of plants for diabetes (Swanston-Flatt et al., 1989) and on plants and phytochemicals

poglycaemic effects have been scientifically investigated (Perl, 1988; Handa et al., 1989; Day, 1990; Marles and Farnsworth, 1995).

The unripe fruit and seeds of *Momordica charantia* L. (Cucurbitaceae) have been the subject of over a hundred scientific articles describing their pharmacological or phytochemical properties. The aim of this review is to summarise the evidence for the anti-diabetic properties of *Momordica charantia*, present its known phytochemical constituents and discuss the possible correlation between the two.

Habitat and traditional uses

The native country of *Momordica charantia* is uncertain, but the plant is cultivated throughout the tropics, particularly in India, China, East Africa and Central and South America (Walters and Decker-Walters, 1988). It is occasionally grown as an ornamental creeper, but more commonly cultivated for use of the unripe fruit as a vegetable. The fruit has a number of different local names – bitter gourd, bitter-melon, balsam-pear, undeamor (South America), karela (India), cattilla or go-lah (Jamaica); the reported spelling of the local names is often variable. The wild variety (*M. charantia* var. *abbreviata*) grows as a weed in the West Indies, where the plant is known as cerasee (Jamaica) or sorossie (Dominican Republic). This variety has smaller fruit than the Indian one. The term karela is used throughout this review to denote all varieties of the fruit since in the majority of studies the type used has not been specified.

In addition to its major use as an anti-diabetic agent, karela has been used in India and Sri Lanka as a tonic, emetic and laxative (Nadkarni, 1982). Both the cultivated and wild forms are used for this purpose (Bailey et al., 1986). In South/Central America, cerasee fruit or tea (see below) is used for diabetes, colds and fevers, stomach aches, constipation in children and the induction of abortion (Arvigo and Balick, 1993; West et al., 1971). Traditional Chinese uses for the fruit, seeds, vines and leaves include gastroenteritis, diabetes, tumours and some viral infections (Zhang, 1992a).

When used as an anti-diabetic remedy, karela juice prepared by crushing and straining the unripe fruit (ca. 50 ml) is taken once or twice a day. Fried karela may also be consumed. Cerasee on the other hand, is taken as a decoction or "tea" (hot water extract) of the aerial parts of the plant, free of large fruit (Bailey et al., 1986).

Studies in human subjects

Karela fruit. To date, no large scale clinical trial has been reported on the anti-diabetic effects of karela, but a number of studies using small groups of diabetic patients have been conducted. Both non-insulin-dependent diabetes mellitus (NIDDM, Type II, maturity onset) and insulin-dependent (IDDM, Type I, juvenile onset) patients have participated.

Lau et al. (1987) have described some early studies (1950–1974) carried out in India and the Caribbean, in which karela's anti-diabetic activity was observed. More recent interest was aroused when Aslam and Stockley (1979) reported a case of a possible interaction, in the form of decreased glycosuria, between a curry containing karela and the anti-diabetic drug chlorpropamide taken by an Asian NIDDM patient. Following this, Leatherdale et al. (1981) carried out a study in 9 Asian NIDDM outpatients living in the United Kingdom. Acute administration of karela juice with a glucose load resulted in a significant improvement in glucose tolerance without increasing the insulin levels in the blood. Daily consumption of fried karela for 8 to 11 weeks had a similar, though not statistically significant, effect. Nevertheless, there was a significant reduction in glycosylated haemoglobin, indicating an improved control of blood glucose levels over this period.

Further evidence for a beneficial chronic effect is that an improvement in both glucose tolerance, and fasting blood glucose levels was observed in 8 NIDDM patients following 7 weeks of daily consumption of powdered karela fruit (Akhtar, 1982). Srivastava et al. (1993) reported that 3–7 weeks treatment of diabetics with powdered fruit led to a mean fall of 2.5% (range 0.4–4.8%) in post-prandial blood glucose levels. There was a marked fall in both blood and urine [illegible] over 7 weeks in a prominent anti-diabetic aqueous extract of the fruit. Glycosylated haemoglobin showed a significant reduction by the end of the trial.

By contrast, Kuti et al. (1982) reported that whole karela (acute or chronic) resulted in a reduction in glycosuria, there was no effect on blood glucose. [illegible] after the administration of karela extract and [illegible] any effects of the fruit may have diminished. The author with all Leatherdale et al. (1981), suggested that improved glucose tolerance is most marked within the first 20 minutes of karela administration. Inter-patient variation may also explain a poor response to karela. Welihinda et al. (1986) reported that karela juice significantly improved glucose tolerance in only 10 of the 18 patients tested.

P-insulin. In 1974, Khanna et al., isolated a polypeptide (p-insulin or v-insulin) from karela. A significant hypoglycaemic effect was observed in 6 IDDM, 1 NIDDM and 2 asymptomatic diabetics administered p-insulin subcutaneously (Balildwa et al., 1977). In a later study by Khanna et al. (1981) subcutaneous p-insulin led to a significant fall in blood glucose in 11 IDDM patients, whereas a similar effect in 8 NIDDM patients did not reach statistical significance. One IDDM patient was reported to have been maintained on p-insulin for 5 months with no complaints of side effects.

Karela seeds. Oral administration of [illegible] seeds produced a significant reduction [illegible]

blood sugar values in 14 NIDDM and 6 IDDM patients (Grover and Gupta, 1990).

In vivo studies in laboratory animals

Studies using laboratory apimals have included normal animals of various species, and those in which diabetes mellitus has been induced by administration of alloxan or streptozotocin (STZ). These two drugs are known to selectively damage beta cells of the pancreas, resulting in partial or virtual loss of insulin production (Fischer, 1985).

Karela juice or extracts. The three main animal species in which the effects of karela juice or karela solvent extracts have been investigated are the rabbit, rat and mouse.

Rabbit model. One of the earliest reports of karela's activity was by Sharma et al. (1960) who reported that the juice caused an improvement in glucose tolerance in alloxan diabetic but not normal animals. Somewhat in contrast to this, Akhtar et al. (1981) found that dried karela fruit caused a significant dose dependent decrease in blood glucose and that a higher minimum dose was required for alloxan-treated rabbits than normal ones. However, Kulkarni and Gaitonde (1962) saw no reduction in fasting glucose levels on either acute or chronic administration of dried karela juice to normal rabbits. These conflicting results may be due to variations in blood sampling times and dosages of both karela and alloxan.

A number of solvent extracts of karela have also been tested. Intravenous administration of a chloroform soluble extract of the juice resulted in a marked hypoglycaemic effect in alloxan-treated but not normal rabbits (Tiangda et al., 1987). This may be an indication of greater pancreatic β-cell sensitivity to karela in alloxan treated animals. Glucose tolerance in alloxan recovered rabbits was improved by oral administration of a benzene extract of karela, but not an ethanolic one (Venkama Babu et al., 1988). Three non-sapogenic hypoglycaemic and 1 hyperglycaemic principles were reported to have been isolated from karela, but their identities were not given.

Rat model. Rat models have been widely used to study the effects of karela juice and its extracts. Improved glucose tolerance on acute administration of the juice has been demonstrated in normal rats (Karunanayake et al., 1984; Chandrasekar et al., 1989) and in rats with anterior pituitary extract-induced hyperglycaemia (Gupta, 1963). Chronic administration over 30 days lowered the mean glucose tolerance in a group of STZ-treated rats, but this did not reach statistical significance (Karunanayake et al., 1990).

Higashino et al. (1992) found that a polar solvent extract of karela improved tolerance of both orally and intraperitoneally administered glucose, suggesting that a mechanism involving impaired glucose absorption from the gastro-intestinal tract was not involved. Ali et al. (1993a) demonstrated that improved glucose tolerance only occurred in NIDDM-model STZ-treated rats and not those in which IDDM had been induced with a higher dose of STZ. This suggests an insulin secretagogue activity by karela. However, Leatherdale et al. (1981) found no significant increase in insulin levels in response to karela treatment in normal rats.

As well as improved glucose tolerance, a hypoglycaemic effect on acute administration of karela juice in fasted rats has been demonstrated in both normal (Leatherdale et al., 1981; Karunanayake et al., 1984; Chandrasekar et al., 1989) and STZ-treated animals (Higashino et al., 1992). However, Ali et al. (1993) found that very high doses of STZ can abolish the effect of karela. In alloxan induced diabetic rats, chronic administration of karela for 20 days was found to lower blood glucose significantly in a dose dependent manner (Srivastava et al., 1987, 1988, 1993). However, Platel et al. (1993) found that 8 week administration of freeze-dried fruit to normal animals did not affect blood glucose levels, possibly due to the operation of normal homeostatic mechanisms. Karela juice administered prior to alloxan did not protect the animals from the induction of hyperglycaemia (Sharma et al., 1960).

Other potentially beneficial effects of karela administration include lowering of serum cholesterol in normal rats (Platel et al., 1993) and delayed cataractogenesis in STZ-diabetic animals (Srivastava et al., 1987, 1988, 1993).

Mouse model. In normal mice, treatment with karela extracts resulted in improved glucose tolerance using either orally or intraperitoneally administered glucose. However, no significant difference observed between insulin levels in treated and control animals (Way et al., 1990). A hypoglycaemic effect of the juice in STZ-treated animals was also demonstrated. The results of fractionation studies implied the presence of more than one active component, possibly alkaloidal in nature (structure not given).

P-insulin. Subcutaneous administration of a polypeptide, p-insulin, isolated from karela to fasted gerbils and langurs caused a significant fall in blood glucose (Khanna et al., 1981).

Charantin. Charantin (Fig. 1), a mixture of sitosterol and stigmastadienol glucosides was isolated from karela by Lothikar and Rajarama Rao (1960/61) in approximately 0.01% yield. A decrease in blood glucose concentration was found when charantin was administered to fasted normal rabbits orally or intravenously. However, the data was obtained using only one or two animals at each dosage level and no controls were carried out. In a more elaborate study (Lothikar and Rajarama Rao, 1966), charantin administered to normal rabbits intravenously or orally produced a gradual but significant fall in blood sugar. In alloxan diabetic rabbits, the effects were more erratic. Pancreatectomy was found to reduce but not abolish the hypoglycaemic effect of charantin (Lothikar and Rajarama Rao, 1966), indicating a dual mechanism of action.

Karela seed. As in human subjects (Grover and Gupta,

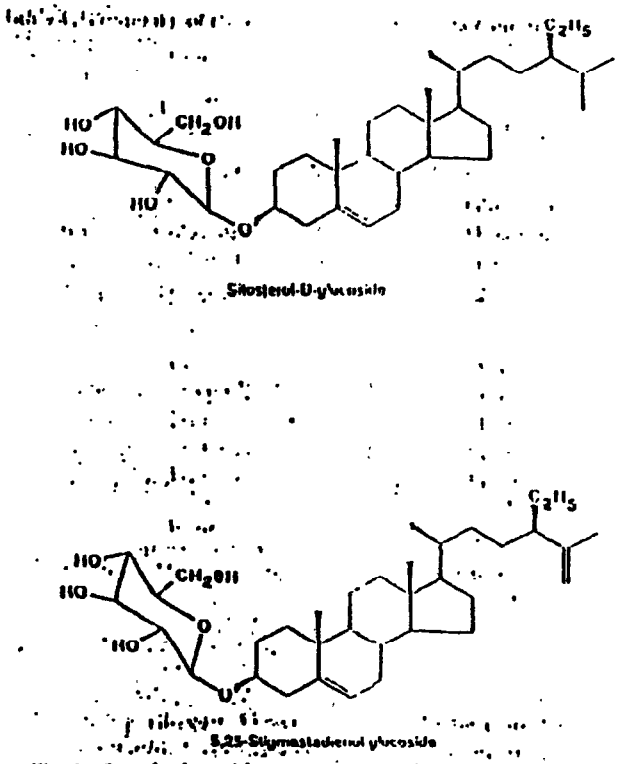
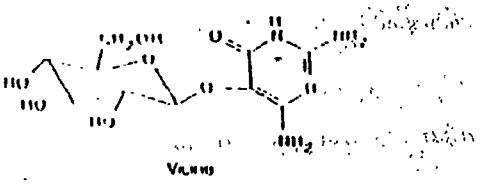


Fig. 1. Sterol glucoside components of charantin isolated from *Momordica charantia* fruit.



Vicine

Fig. 2. Vicine, a putative hypoglycaemic compound from *Momordica charantia* seeds.

1990), karela seed was found to lower blood glucose levels in STZ induced diabetic rabbits (Kedar and Chakrabarti, 1982). The seed also reversed low muscle and liver glycogen and the elevated serum cholesterol, fatty acids and triglycerides induced by STZ. Polar solvent (methanol, 50% aqueous ethanol, normal saline) extracts of karela seed showed a significant hypoglycaemic effect in fasted albino rats (Dubey et al., 1987). The methanol and saline extracts were also able to reduce adrenaline-induced hyperglycaemia. In both cases, the methanol extract was the most potent. However, Ali et al. (1993a) reported that a methanolic extract of the seed did not reduce blood glucose levels in fasting or post-prandial states in normal and STZ-treated IDDM rats.

Vicine. Vicine (Fig. 2) has been isolated from the seeds of karela in 0.6% yield (Handa et al., 1990). Intraperitoneal administration of vicine caused a hypoglycaemic response in normal fasting albino rats. The dose used was equivalent to about five times the amount of seed administered orally by Kedar and Chakrabarti (1982) to obtain a response.

Karela vines and aerial parts. Cerasee tea (prepared from the vines) was found to lower basal glucose concentrations and to improve glucose tolerance in normal mice (Bailey et al., 1985), with no significant change in the plasma insulin level. A hypoglycaemic response was also observed in STZ-treated mice. When cerasee tea was substituted for drinking water for 12 days, glucose tolerance measured on day 13 was improved. More recently, Ali et al. (1993a) tested methanolic and saponin-free methanolic extracts of the whole plant of *Momordica charantia* in normal rats. No effects were seen on fasting blood glucose.

Effects on tissues and enzymes; possible mode of action

Attempts have been made to obtain further information on the mode of action of karela fruit and seeds through experiments using enzymes, tissues or cells *in vitro* or examining organs isolated from karela treated animals. Karela fruit and seed preparations have a number of biological effects *in vitro* (Table 1), which may give an indication of their mode of action.

Glucose absorption. A theoretical means by which glucose tolerance can be improved is by decreased absorption of glucose from the gut. Ner and Yaniv (1985) reported that glucose up-take by everted gut was inhibited in the presence of extracts of karela fruit. However, from the *in vitro* work of Day et al. (1990) and Ng et al. (1992), it would appear that this is not the mechanism involved in the action of karela since tolerance of an orally administered glucose is also improved. There have been no studies reported to date on effects of karela on enzymes involved in the digestion of carbohydrates, e.g. α-amylase.

Insulin secretion. In a number of *in vitro* studies (Welihinda et al., 1982a, b; Ali et al., 1993b; Hosibuzzaman et al., 1994), extracts from the fruit have been found to stimulate insulin release from isolated pancreatic islet cells. The responsiveness of STZ and alloxan treated animals to karela, would seem to suggest that pancreatic stimulation is not involved. However it must be noted that STZ and alloxan treatments may not result in complete destruction of pancreatic β-cells. For instance, in the study by Kedar and Chakrabarti (1982), STZ-treated animals were responsive to glibenclamide, which acts by stimulation of insulin release from the pancreas. In addition, Ali et al. (1993a) found that the effects of karela could be abolished by treating the animals with a higher dose of STZ. In some studies (Sharma et al., 1960; Tiangda et al., 1987), alloxan treated rabbits were more responsive to karela extracts than normal animals. This may indicate a sensitization of the β-cells

Table 1. Summary of the *in vitro* effects of karela fruit and seed extracts.

Plant part	Extract	Process studied	Effect	Reference
Fruit	Ethanol, water	Glucose uptake into inverted gut	Inhibited	(1)
Fruit	Water	Insulin release from isolated islets	Stimulated	(2,3,4,5)
Fruit	Juice	Gluconeogenesis in kidney slices	No effect	(6)
Fruit	Ethanol	Gluconeogenetic liver enzymes	Depressed*	(7)
Fruit	Ethanol	Glucose oxidation in liver	Inhibited	(1)
Fruit	Ethanol	Hexokinase (yeast)	Inhibited	(1)
Fruit	Ethanol	Hepatic glucose-6-phosphate dehydrogenase	Raised*	(7)
Fruit	Juice	Tissue respiration in diaphragm muscle	No effect	(6)
Fruit	Juice	Glucose uptake into diaphragm muscle	Stimulated	(6)
Fruit	Juice	Glycogen content in liver and muscle	Increased*	(6)
Fruit	Juice	Oxygen radical scavenging	Activity present	(8)
Fruit, seed	Ethanol, saline, water	Lipogenesis in adipocytes	Stimulated	(9,10)
Fruit, seed	Ethanol, saline, water	Hormone induced lipolysis in adipocytes	Inhibited	(9,11,12)
Seed	Proteins, low MW species	Lipogenesis in adipocytes	Stimulated	(10,13)
Seed	Lectins, saponin	Lipolysis in adipocytes	Inhibited	(11,12, 14)
Fruit	Juice	Triglyceride content in adipocytes	No effect	(6)
Seed	Proteins	Adrenal steroidogenesis	No effect	(15)

* Karela juice/extract administered *in vivo* prior to removal of tissue for analysis.
References: (1) Meir and Yaniv (1985); (2,3) Welihinda et al. (1987 a,b); (4) Ali et al. (1993b); (5) Mowshumzaman et al. (1994); (6) Welihinda and Karunanayake (1986); (7) Shibib et al. (1993); (8) Rao (1991). (9,10) Ng et al. (1985, 1986 a); (11,12) Wong et al. (1985 a,b); (13,14,15) Ng et al. (1987 a, 1986 b, 1987 b).

to karela by alloxan. However, it should be noted that increased insulin levels have not been observed in karela treated mice (Day et al., 1990), rats or humans (Leatherdale et al., 1981) *in vivo*.

Insulinomimetic effects. Karela juice shows certain insulinomimetic effects such as increased glucose uptake into muscle, stimulation of lipogenesis, and inhibition of lipolysis on tissue preparations *in vitro* (Table 1). *In vitro* tests on tissues taken from animals treated with karela have also shown a depression of hepatic gluconeogenetic enzymes, and increased liver and muscle glycogen.

There is conflicting data on effects of karela extracts on tissue respiration. Welihinda and Karunanayake (1986) found that karela juice did not show any effect on tissue respiration by diaphragm muscle *in vitro*. However, Meir and Yaniv (1985) reported that karela inhibited the oxidation of glucose by liver tissue, possibly at the first step in glycolysis i.e. phosphorylation by hexokinase. These contradictory results may be due to differences in the tissue, methodology and type of karela preparation used. A more reliable indicator of effect of karela on tissue respiration may be that demonstrated by Shibib et al. (1993). Liver glucose-6-phosphate dehydrogenase (G-6-PDH) activity was elevated on *in vivo* administration of karela ethanolic extract by gastric intubation. This would enhance the utilisation of glucose by the liver leading to a lowering in blood glucose.

The lipogenic and anti-lipolytic effects of karela juice *in vitro* are shared by seed extracts. A saponin (not identified) and proteins have been found to account for the *in vitro* effects of the seeds. The proteins are believed to be lectins; the abortifacient proteins α- and β-momorcharin also found in the seeds are not active in this assay (Wong et al., 1985 a, b). However, against this, Welihinda and Karunanayake (1986) reported that adipose tissue of karela treated rats did not differ significantly in triglyceride content from that of control animals.

Thus, inhibition of glucose absorption, insulin secretagogue activity and insulinomimetic effects have been attributed to karela in *in vitro* tests. However, not all of these have been fully supported by *in vivo* data, probably due to the compounds showing activity *in vitro* not being bioavailable *in vivo*.

Phytochemicals isolated from *Momordica charantia* and their relationship to its anti-diabetic effects

Since the early 1960's a number of phytochemicals have been isolated from *Momordica charantia* fruit, seeds and whole plants. A review of the known constituents was published in 1989 (Fiche Espèce, 1989). This data and supplementary information are given in Tables 2–4. In some cases biological activities, such as insulinomimetic properties, protein synthesis inhibition, or insect attractant effects have been associated with the pure compounds or with fractions rich in a particular type of compound eg saponins or proteins. Possible identities which emerge for the hypoglycaemic principle in *Momordica charantia* are steroidal glycosides, insulinomimetic lectins and alkaloids: the evidence relating to each of these is discussed below.

Steroidal glycosides. The earliest reported active constituent of karela fruit was "Charantin" (Fig. 4), a mixture of glucosides of sitosterol and 5,25 stigmastadien-3 β-ol (Lot-

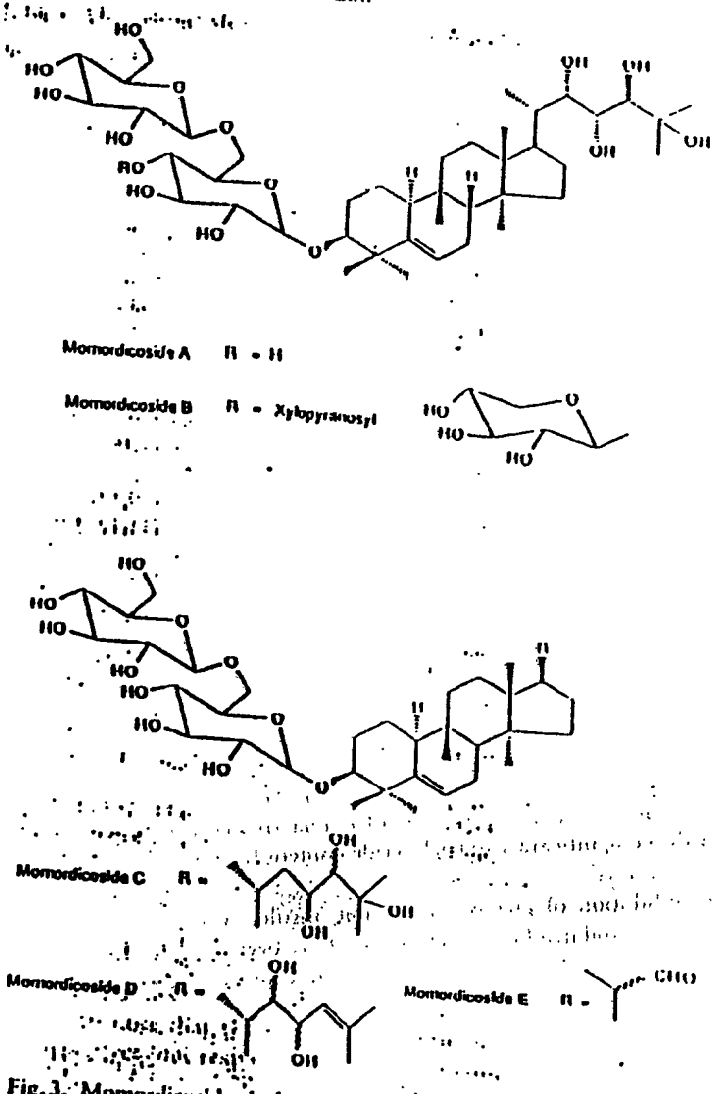


Fig. 3. Momordicosides isolated from *Momordica charantia* fruit and seeds.

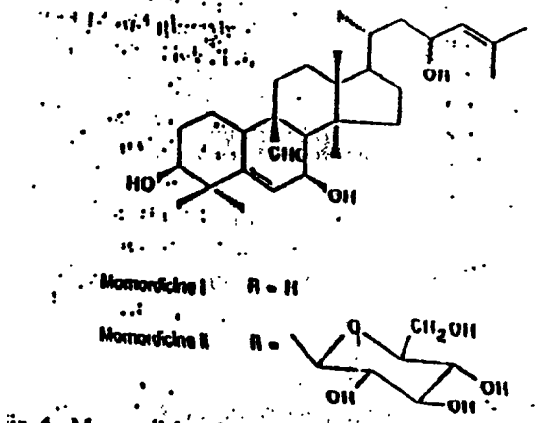
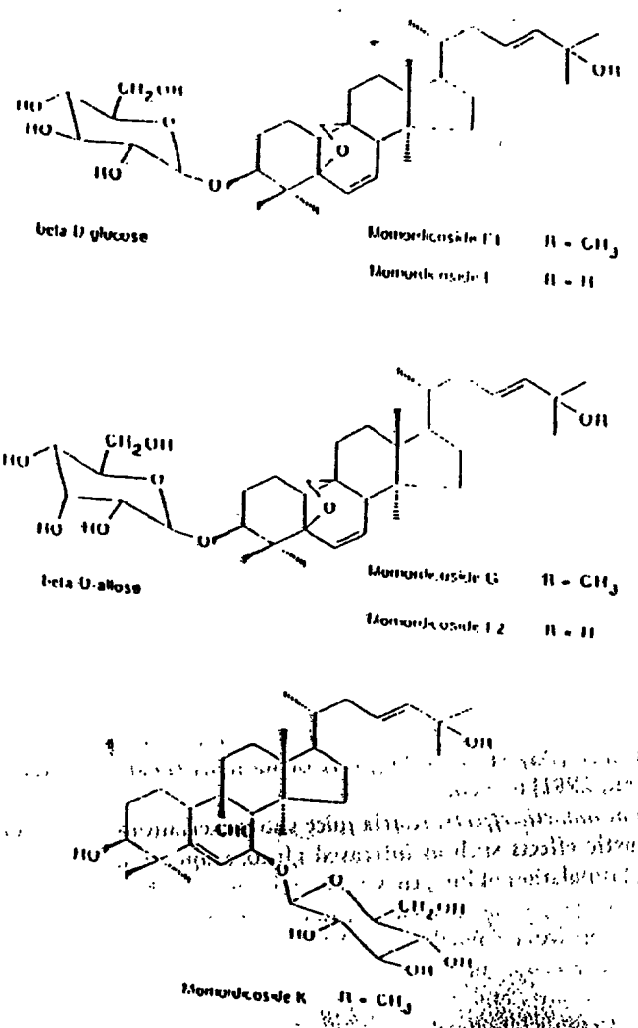


Fig. 4. Momordicines isolated from *Momordica charantia* leaves.



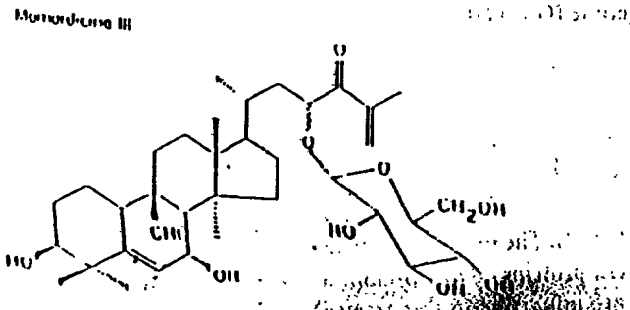


Table 2. Phytochemicals isolated from *Momordica charantia* fruit.

Phytochemical	Comment	Reference
Steroids		
Charantin (Figure 1)	Mixture (1:1) of sitosterol and stigmastadienol glycosides. Hypoglycaemic in rabbits on oral or intravenous administration.	Sucrow (1965, 1966) Lotlikar and Rajarama Rao (1960/61; 1966)
	Does not stimulate insulin release from pancreatic cells *in vitro*.	Welbinda et al. (1982 a)
Momordicosides (Figure 3)	G, F_1, F_2, I – non bitter cucurbitacin glycosides.	Okabe et al. (1982 a, b)
	K, L – bitter cucurbitacin glycosides.	Okabe et al. (1982 b, c)
Acylglucosylsterols	Antimutagenic against mitomycin C in mouse.	Guevara et al. (1989)
Linolenoylglucopyranosylclerosterol	Ripe fruit; attractant for *Dacus cucurbitae* insect.	Sato and Kato (1987 a)
Amino Acids	Ripe fruit.	Dhalla et al. (1961)
Lipids		
Fatty acids	Most abundant (45%) is α-eleostearic acid.	Yuwai et al. (1991)
Galactopyranosyldilinolenoylglycerol	Attractant for *Dacus cucurbitae*.	Sato and Kato (1987 b)
Phenolic compounds	12 phenolic acids and flavonoids reported.	Venkataramaiah and Rao (1983)
Proteins		
P-insulin, V-insulin	11 k Dalton; hypoglycaemic in man and animals (parenteral).	Khanna et al. (1981)
Acid ethanol fraction	Stimulates lipogenesis; inhibits lipolysis in adipocytes *in vitro*.	Ng et al. (1985)
Acid acetone powder	Inhibits lipolysis in adipocytes *in vitro*.	Wong et al. (1985 b)
Guanylate cyclase inhibitor	Found only in ripe fruit; inhibits the enzyme in rat tissue.	Vesely et al. (1977)
	Inhibits growth of prostate adenocarcinoma.	Claflin et al. (1978)
Cytostatic factors (ripe fruit)	11 k and 70 k Dalton; cytostatic to leukaemic lymphocytes but not normal ones; inhibits RNA, DNA and protein synthesis.	Takemoto et al. (1982 a, b)
Anti-lymphoma factor (ripe fruit)	40 k Dalton; produces transferable resistance to lymphoma in mice. Lymphocytes from treated mice more sensitive to concanavalin A.	Takemoto et al. (1984) Cummick et al. (1984)
Uncharacterised compounds		
Alkaloid fraction	Slow acting hypoglycaemic effect in fasted STZ rats.	Day et al. (1990)
Kakra 1b, 11 a, 11 b	Non-steroidal hypoglycaemic agents.	Srivastava et al. (1993)
Anionic substance	From ripe fruit; M Wt about 150; inhibits tubulin polymerisation.	Lowe et al. (1983)
Buffer extract	Inhibits tumour formation; possible immunostimulant.	Jilka et al. (1983)

likar and Rajarama Rao, 1960/61, 1966). However, it is important to note that the dose of charantin required to elicit a hypoglycaemic response in rabbits was equivalent to 180 to 315 g of fruit orally and 81 g fruit intravenously, whereas a hypoglycaemic effect can be seen in rabbits with about 10 to 15 g of the fruit per kg body weight.

In 1975, Olaniyi isolated a substance "foetidin", from the whole plant of *Momordica foetida*, which was found to be identical in composition to charantin. Marquis et al. (1977) claimed that at 18 hours from administration, foetidin lowered blood glucose in fasting rats in an effect comparable to insulin. This claim is often quoted in the literature as support that the steroidal mixture is the active principle of *Momordica charantia*. However, a closer examination of the original data presented in the paper shows that foetidin was not significantly different from control at time points other than the 18 hour sample.

It is now known that *Momordica charantia* fruit, seeds and vines contain other steroidal glycosides (momordicosides and momordicines; Tables 2–4, Fig. 3 and 4). A saponin fraction from the seeds of karela showed insulinomimetic effects *in vitro* (Wong et al., 1985 b; Ng et al., 1986 b). The contribution of steroidal constituents other than charantin to the *in vitro* anti-diabetic effects of *Momordica charantia* has not been evaluated.

Insulinomimetic proteins. *In vitro* insulinomimetic effects have been observed with fruit proteins (Table 2) and seed proteins (Table 3). The active seed protein is believed to be a galactose binding lectin (Table 3). Khanna et al. (1981) reported that an 11 k Dalton protein (p-insulin or v-insulin) caused hypoglycaemia in man and laboratory animals on parenteral administration.

Proteins are generally considered to be inactive when administered by the oral route, as they would undergo extensive digestion by proteolytic enzymes. Thus the possibility of a polypeptide being responsible for the hypoglycaemic effects of the fruit or seeds when given orally must be viewed with some scepticism. However, against this, there is some evidence (Pusztai, 1986) that lectins may be absorbed into the bloodstream from the gastro-intestinal tract. Khanna (1985) has stated without any supporting data that p-insulin is also effective orally.

Alkaloids. Day et al. (1990) reported that hypoglycaemic activity of fractionated karela fruit juice resided in an alkaloid-rich fraction. The alkaloids have not been isolated or characterised. The pyrimidine nucleoside, vicine (Fig. 2) has

Table 3. Phytochemicals isolated from *Momordica charantia* seeds.

Phytochemical	Comment	Reference
Proteins		
Galactose binding lectins	Lectins I and II (both 26 k Dalton); only I is a haemagglutinin.	Li (1980)
	Momordica agglutinin (32 k Dalton); haemagglutinin.	Lin et al. (1978)
	Momordica charantia lectin (MCL) 115 k Dalton in size; inhibits protein synthesis and inactivates ribosomes.	Barbieri et al. (1979, 1980)
	Lectin of 120 k Dalton size (MCL?); two binding sites and haemagglutination inhibited by galactose and derivatives.	Mazumder et al. (1981); Khan et al. (1981)
	Lectin of 124 k Dalton size (MCL?); insulinomimetic effects	Ng et al. (1986 c, 1987 b)
Insulinomimetic extracts	Lectin (MCL?); antilipolytic in adipocytes.	Wong et al. (1985 b)
	Saline extract; antilipolytic in adipocytes.	Wong et al. (1985 a, b)
	Acid ethanol extracts; lipogenic and antilipolytic in adipocytes.	Ng et al. (1985, 1986 a)
	Insulinomimetic peptides (8 k Dalton).	Ng et al. (1987 a)
Ribosome inactivators	*Momordins*	
	Tumour protein synthesis inhibitor (24 k Dalton); haemagglutinin.	Lin et al. (1978)
	Momordins a and b (28 k Dalton) isolated.	Minami et al. (1992)
	Amino acid sequence of momordins reported.	Minami and Funatsu (1993)
	Momordica charantia inhibitor (MCI)	
	Protein synthesis inhibitor (23 k Dalton); not haemagglutinin.	Barbieri et al. (1980)
	Glycoprotein with pI 8.60.	Falasca et al. (1982)
	Immunosuppressive.	Spreafico et al. (1983)
	Known site of interaction with eukaryotic rRNA.	Endo et al. (1988)
Abortifacients	Saline extract abortifacient fraction M' II	Shin et al. (1985)
	Terminates pregnancy in rats; trophoblasts necrosed.	Wong et al. (1985 a)
	Non-haemagglutinins; no antilipolytic effects in adipocytes.	
	Momorcharins (α, β)	
	α (29 k Dalton) and β (28 k Dalton) forms equipotent in mice.	Yeung et al. (1986)
	β inhibits growth of mouse embryos and endometrial cells.	Chan et al. (1985)
	Non-haemagglutinins; no antilipolytic effects in adipocytes.	Wong et al. (1985 b)
	Immunosuppressants; tumour growth inhibitors; lyse DNA.	Go et al. (1992)
	Toxic to hepatocytes *in vitro*.	Ng et al. (1994)
Antivirals	MCI inhibits multiplication of herpes simplex virus I.	Pan-Tamura et al. (1992)
	Momorcharins (α, β) inhibit HIV replication.	Lee-Huang et al. (1990)
	MAP 30 (30 k Dalton) inhibits HIV infection and replication.	Lee-Huang et al. (1990)
Miscellaneous	2 storage proteins (4 x 5.5 k Dalton subunits).	Li (1977)
	Anticancer fractions CAP-II; inhibits sarcoma growth in mice.	Yau et al. (1985)
	Ribonuclease Mc (21 k Dalton) differs from fungal RNAses.	Ide et al. (1991)
	MCII A, B, C (30 k Dalton); trypsin inhibitors.	Huang et al. (1992)
Nucleosides		
Ricine (Figure 2)	Isolated from seeds; may be linked to favism.	Dutta et al. (1981); Barron et al. (1982)
	Hypoglycaemic in rats; non-haemolytic to sheep erythrocytes.	Handa et al. (1980)
Zeatin + riboside	Cytokinins found in immature seeds.	Iyer et al. (1981)
Amino Acids	13 amino acids reported including γ-amino butyric acid.	Basu et al. (1982)
Fatty Acids	6 fatty acids reported including eleostearic acid.	Lakshminarayana et al. (1982)
Terpenoids	15 sterols and 3 pentacyclic triterpenoids from two seed varieties.	Ishikawa et al. (1986)
Steroidal Glycosides		Kikuchi et al. (1986)
Saponin fraction	Haemolytic; inhibits hormone induced lipolysis in adipocytes.	Wong et al. (1985 b)
	Inhibits glucose incorporation into lipids in adipocytes.	Ng et al. (1986 b)
Momordicosides (Figure 3)	A and B.	Okabe et al. (1980)
	C, D and E.	Miyahara et al. (1981)

Table 4. Phytochemicals isolated from *Momordica charantia* whole plants, vines or leaves.

Phytochemical	Comment	Reference
Steroidal Compounds		
Saponin	Uncharacterised; non-haemolytic; from whole plant.	Rivera (1941)
Sterols and steroidal glycosides	Stimastenol; stigmastadienol and stigmastadienol glucoside from leaves.	Ulubelen and Sankawa (1979)
	Cucurbita-5,24-dienol (plant part not stated).	Fiche Espèce (1989)
	Sitosterol and sitosterol glucoside from aerial parts of plant; not anticonvulsant or anti-inflammatory.	Lal et al. (1990)
	Momordicines I, II and III (Figure 4); bitter glycosides from leaves.	Yasuda et al. (1984)
	Momordicine II feeding deterrent to red pumpkin beetles.	Chandravadana (1987)
	3 Cucurbitane triterpenoids (not momordicines) from leaves.	Fatope et al. (1990)
Alkaloids:	From whole plant; white precipitate with Mayers reagent.	Rivera (1941)
(uncharacterised)	From whole plant; dose dependent anti-inflammatory effect.	Lal et al. (1990)
	Tertiary alkaloids in alcohol extract of leaves.	Ulubelen and Sankawa (1979)
Amino Acids	γ-Amino butyric acid; may be hypotensive principle.	Durand et al. (1962)
Proteins	Guanylate cyclase inhibitor from leaves (and ripe fruit).	Vesely et al. (1977)
Long-Chain Compounds	Hentriacontanol.	Lal et al. (1990)
	Triacontanol; n-octosan; phytosphingosine.	Ulubelen and Sankawa (1979)

been isolated from the seeds (Dutta et al., 1981; Barron et al., 1982). This "alkaloid" has been found to induce hypoglycaemia in rats at an intraperitoneal dose equivalent to 16 g of seeds per kg body weight (Handa et al., 1990). Kedar and Chakrabarti (1982) found the seed powder to be orally effective in rabbits at 1–3 g per kg body weight. Thus vicine may not account for all the activity of the seeds.

Kakra compounds. Srivastava et al. (1993) isolated three non-steroidal hypoglycaemic compounds (Kakra 1 b, 111 a and 111 b) from the fruit which differ from earlier reported principles, ie. p-insulin or charantin. The structure of these compounds was not elucidated.

Other pharmacological and toxicological properties

A number of effects of *Momordica charantia* unrelated to diabetes have been investigated. No data is available on standard toxicity parameters e.g. LD_{50} values of the juice, seeds or plants. However some information on toxicity is available from observations made during experimental or clinical use of *Momordica charantia* extracts in animals or humans.

Anti-cancer. Protein fractions obtained from the fruit and seed of *Momordica charantia* have the ability to inhibit cell growth, guanylate cyclase activity and ribosomal activity (Table 2, 3). West et al. (1971) demonstrated inhibitory effects of whole plant extracts on seedling root growth, division of fertilised sea urchin eggs, rat foetal growth (if injected on day of mating) and the growth of Hep_1 cells in culture. They also report a single case study of a leukaemia patient in whom regular intake of the extract led to a fall in white blood cell count, and an increase in blood haemoglobin.

Antiviral. The growth of herpes simplex virus I (Foa Tomasi et al., 1982) and human immunodeficiency virus I (Lifson et al., 1988; Lee-Huang et al., 1990) is inhibited by karela extracts. Increased T-cell count and a normalisation of the CD 4/CD 8 ratio seemed to occur in three HIV positive patients given regular doses of karela juice (Zhang, 1992 b). The juice was administered as a retained enema i.e. rectally. This may explain its apparent effectiveness since the active anti-viral components of *Momordica charantia* are believed (Zhang, 1992 b) to be the proteins α and β-momorcharin and MAP (Table 3), which would be expected to undergo hydrolysis by pancreatic enzymes if administered by the oral route.

Analgesic effects. A methanolic extract of the seeds from unripe fruit has been shown to produce a marked dose-dependent analgesic effect in mice and a much weaker effect in rats (Biswas et al., 1991), but using different test systems for the two species. Naloxone pretreatment failed to modify the analgesic response, suggesting that opioid receptors were not involved.

Anti-inflammatory effects. A dose related anti-inflammatory effect has been demonstrated using carageenin-induced rat hind-paw oedema (Lal et al., 1990). Free radical scavenging activity of the juice *in vitro* (Rao, 1991) may be involved.

Hypotensive action. "Cerasee" (aerial parts of *Momordica charantia*) extract showed a marked transient depressor effect on injection to the anaesthetised dog (Feng et al., 1962). Gamma amino butyric acid has been suggested to be responsible for this effect (Durand et al., 1962).

Antifertility effects. Oral administration of karela fruit extract (1.75 g/day for 60 days) to male dogs resulted in testicular lesions and mass atrophy of spermatogenic elements (Dixit et al., 1978). Serum enzymes were normal implying that an infertility state was induced without altering general metabolic activity in the animal.

A study by Stepka et al. (1974) found that daily oral administration of the fresh juice of *Momordica* (species not stated) leaves to a group of female mice, reduced the fertility rate. This was reversed on withdrawal of the treatment. No pathological changes were seen in any of the maternal organs, but in some cases, concepti were seen as necrotic masses. In more recent work proteins capable of inducing abortions (α and β momorcharins) and necrosis of placental trophoblasts have been isolated from *Momordica charantia* seeds (Table 3). It is possible that similar proteins occur in the leaves. Uterine bleeding has been induced in pregnant rats given karela juice (6 ml/kg) orally (Zhang 1992 b), while 2 pregnant rabbits given karela juice (6 ml/kg) suffered uterine haemorrhage and death within a few hours (Sharma et al., 1960). No such effect was noted in non-pregnant females.

Effects on growth, blood and serum lipids. Chronic administration of karela extract (1.75 g orally per day for 20–60 days) to dogs resulted in elevated levels of serum cholesterol and non-esterified fatty acids, but no significant changes in body weight or serum enzymes (Dixit et al., 1978). Rats maintained on a diet containing freeze-dried karela for 8 weeks showed no change in food consumption rate or growth rate (Platel et al., 1993). At the end of this period, organ weights (liver, kidney, testes, spleen, adrenals and heart) were similar to those of control animals. Blood cell counts, cell volume and haemoglobin parameters showed no significant difference to controls and remained within the normal range. However in this study, there was a significant decrease in blood cholesterol.

Hepatotoxicity. Following the administration of karela juice and seed extract to rats (10 ml/kg body weight daily for 30 days), serum γ-glutamyl transferase and alkaline phosphatase was significantly elevated, but consistent histopathological defects were not observed in the liver (Tennekoon et al., 1994). Therefore the elevated enzymes could either be due to mechanisms not obvious at the histological level or to enzyme induction. The prevalence of dilatation and/or congestion in the hepatic central veins and associated sinusoids was twice as high in the juice treated group as in the seed extract treated and control groups. Ng et al. (1994) have found that α- and β-momorcharins can induce cytoplasmic blebs and other morphological changes in rat hepatocytes *in vitro*. Secretion of various enzyme markers of cell damage is also raised.

Fatal doses in animals. Continuous single or twice daily oral administration of karela juice (6 ml/kg body weight) to 6 rabbits resulted in 5 animals dying within 5–25 days (Sharma et al., 1960). In an acute effect, pregnant but not normal rabbits, died within a few hours of receiving this dose (Sharma et al., 1960). Rats given karela juice (18–40 ml/kg body weight, by intraperitoneal route) became sluggish and died within 6–18 hours. Zhang (1992 b) reported that pregnant rats died within a few hours of receiving karela juice (6 ml/kg body weight) orally. In normal and alloxan diabetic rats given the same dose daily, 80–90% died within 5–23 days. Abdominal injection of the juice at (15 ml/kg body weight) caused death in 6–18 hours. In rabbits receiving 10 ml/kg orally per day, the majority were reported to have shown toxic effects, although the nature of these effects was not given in the paper.

Toxicity in humans. Although toxicity has been observed in some animal studies, if extrapolated to humans, the relevance of the dose and route of administration must be considered. A dose of 6–10 ml/kg would represent a dose of 400 ml–1000 ml for an adult. The normal adult dose is closer to 50 ml, given orally. There are no published reports of fatal or serious effects in adults at this dose.

Patel et al. (1968) reported that administration of the juice or dried juice powder (equivalent to 250–500 g of the fruit) to diabetic patients led to abdominal pain and diarrhoea. Zhang (1992 b) has used orally or rectally administered fruit juice to treat HIV-positive patients. He reports that there is very low clinical toxicity. A patient who had been given the juice daily for over three years did not show any change in blood chemistry or any other untoward effect. Liver, kidney, heart or blood abnormalities have not been reported in any of Zhang's patients despite long term use of *Momordica charantia* fruit juice.

The only report of a potentially fatal reaction in humans is hypoglycaemic coma induced in two small children (Hulin et al., 1988 a, b). The children aged three and four required urgent medical attention following ingestion of a water extract of *Momordica charantia* leaves and vines. In both cases, the Sorrosi (cerasee) tea had been administered by their mothers early in the morning before any other food was consumed. Between 1–2 hours after ingestion, the children experienced convulsions followed by coma. Blood glucose was in the region of 1 mM (normal range 3.8–5.5 mM). Both patients recovered following treatment.

Conclusion

The fruit, seeds and aerial parts of *Momordica charantia* Linn have been used as an anti-diabetic remedy in a number of areas of the world notably India, Sri Lanka, China and the West Indies. Limited studies on humans have shown

that karela fruit juice reduces fasting blood glucose and improves glucose tolerance on acute administration. Prolonged administration causes a lowering of glycosylated haemoglobin in the blood, and decreases glycosuria and basal glycaemia. The hypoglycaemic and anti-hyperglycaemic effects of karela fruit and seeds have also been demonstrated in animal models. Through evidence from animal and *in vitro* studies, there is support for both insulin secretagogue and insulinomimetic activity of the fruit. However, enhanced insulin levels *in vivo* in response to administration of karela have not been observed.

A wide range of compounds have been isolated from *Momordica charantia* fruit, seeds and vines, notably saponins and proteins. Suggested hypoglycaemic compounds include a polypeptide (p-insulin), a steroid mixture (charantin) and a pyrimidine nucleoside (vicine). However, none of these is fully supported as a sole active constituent by the scientific data available. It is possible that a number of active constituents with a range of biological effects beneficial to diabetes are present in the fruit.

Principal toxic properties of karela juice noted in animals are anti-fertility effects and hepatotoxicity, with death occurring on chronic oral treatment with doses of the order of 6 ml/kg body weight. Pregnant females were particularly susceptible. Encouragingly, similar effects have not been reported in humans despite widespread use of the fruit juice both as a medicinal plant and as a vegetable.

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HYPOGLYCEMIC ACTIVITY OF POLYPEPTIDE-p FROM A PLANT SOURCE[1]

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ABSTRACT.—A hypoglycemic peptide, Polypeptide-p, has been isolated from fruit, seeds, and tissue of *Momordica charantia* Linn (bitter gourd). Amino acid analysis indicates a minimum molecular weight of approximately 11,000 (100 residues). Polypeptide-p is a very effective hypoglycemic agent when administered subcutaneously to gerbils, langurs, and humans.

Insulin used in the treatment of diabetes mellitus has usually been obtained in very low yield from animal pancreas, i.e., one pound of pure insulin per 10,000 animals. Side effects of the animal insulin are well known. Recently, insulin has been synthesized by genetic manipulation in *Escherichia coli*, which is a significant scientific achievement.

A number of indigenous drugs have been tried in the past for the treatment of diabetes mellitus. In the tropical world, fruits of *Momordica charantia* (bitter gourd) have been successfully used by diabetic patients; crude extracts have shown hypoglycaemic activity in rabbits (1–3). Khanna *et al.* (4, 5) were able to isolate an active principle earlier called p-insulin or v-insulin from fruits, seeds, and tissue culture of this plant species (6, 7). When administered subcutaneously to human patients, v-insulin showed a significant blood sugar lowering effect (8).

MATERIALS AND METHODS

TISSUE CULTURE.—Seed coats of *M. charantia* were removed, and the seeds were inoculated on revised Murashige and Skoog's medium (9, 10) supplemented with 1 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% agar. The seeds took 5–6 days to germinate and form seedlings. Organized tissue was established (10) from the whole seedlings and maintained for 12–18 months by frequent subculturings of 6–8 weeks in fresh MS medium. This tissue was harvested after the transfer age of 6 weeks and extracted for its polypeptide-p content.

Fruits, soaked seeds, and tissue samples (100 g each) were crushed separately and then frozen. Each of the frozen samples was dissolved in 10 ml of distilled water, 45 ml of 95% ethanol and 3.6 ml of sulfuric acid (99.5%). The mixture was stirred vigorously (5; 11) for 15–20 min at 25–28° and then homogenized by the addition of 60 ml of distilled water and 250 ml of 95% ethanol separately. After each of the mixtures was filtered, enough ammonium hydroxide (28%) was added to adjust the pH of the filtrate to 3. To each of the filtrates, 1.5 liters of acetone was added till a white flocculent precipitate was formed. These mixtures were kept at 5° for 8–10 hr.

The supernatant from each of the continents was decanted off, and the precipitate was dialyzed in a dialysis membrane (30 DM; Union Carbide Corporation, Chicago, U.S.A.; molecular weight cut-off was 6000); distilled water was used to remove the last traces of salt and other dialyzable impurities until the outside water gave a negative test with barium carbonate. The non-dialyzable fraction was collected, dried and crystallized in a 0.001% solution of zinc acetate in water (12). The excess zinc was removed by washing with ethylenediaminetetraacetic acid (EDTA) solution.

Crystallized material was applied to silica gel G coated and activated glass plates along with a standard sample of bovine insulin. The plates were developed in a solvent mixture of *n*-butanol-acetic acid-water; 12:5:2. When the plates were sprayed with ninhydrin (0.25% in acetone) and heated, a single yellow spot (R_f 0.19), which nearly coincided with that of the standard sample of bovine insulin, was observed.

Disc electrophoresis was carried out (10% SDS Biophore Gel, run in tris buffer, operating pH 0.1; 3% acetic acid in lower cell; 90 V; mA 2.6 per tube; Bromophenol blue tracking dye).

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Samples of the crystallized isolate and bovine insulin were separately prepared with SDS biophore buffer containing dithiothreitol and EDTA, injected, and run for 7 hr. Gels collected from the tubes were stained (0.05% Coomassie Brilliant Blue R-250 in 7% aqueous acetic acid) and washed with 10% acetic acid.

The isolate (25 mg) from each of the samples was hydrolyzed with 6N HCl at 100° for 24 hr and filtered. The filtrate was dried and the residue taken up in 50% ethanol. Two dimensional *tlc* was carried out (silica gel G; solvent system, first: *n*-butanol-acetic acid-water, 5:1:1; second: phenol saturated with water; 0.25% ninhydrin in acetone as spraying reagent), and seventeen amino acids were resolved. The isolates were also run in an automatic amino acid analyzer separately (table 1).

TABLE 1. Amino acids of Polypeptide-p of *Momordica charantia* analyzed by automatic analyzer.

Amino acid	μ moles/mg*	Molecular number
Aspartic acid	0.273	17
Threonine	0.138	8.7
Serine	0.195	12
Glutamic acid	0.305	19
Proline	0.159	10
Glycine	0.225	19
Alanine	0.240	15
Valine	0.174	11
½ Cysteine	0.058	3.6
Methionine	0.031	2
Isoleucine	0.110	7
Leucine	0.207	13
Tyrosine	0.016	1
Phenylalanine	0.082	5
Histidine	0.066	4
Lysine	0.200	13
Arginine	0.161	10
NH₃	0.431	(27) omit
TOTAL		160 residues
Approximate molecular weight 11000		

*Volume used 0.46 ml (0.81 mg).

A derivative of the crystallized material (polypeptide-p-$ZnCl_2$) was prepared in the same manner (12) as bovine insulin (Insulin-$ZnCl_2$). Doses of polypeptide-p and polypeptide-p-$ZnCl_2$ (in 0.9% NaCl as the vehicle) were prepared (1.8 mg/ml equivalent to 40 units) as used in the case of bovine insulin. Immunoassays were also carried out.

PHARMACOLOGICAL TRIALS.—Pharmacological studies involved *Meriones hurrianae* Jerdon (gerbils), males and females, and *Presbytis entellus entellus* Dufresne (male langurs). 105 gerbils, weighing 63–87 g were used in the present work. The animals were divided into groups of five each and all were fasted for 12 hr before the beginning of the experiment. These animals were provided with water *ad libitum*. Polypeptide-p-$ZnCl_2$ (0.5 unit/kg in 0.9% NaCl) was administered subcutaneously. Thirty-five animals were injected with an equal amount of 0.9% saline vehicle. The blood samples were obtained through cardiac puncture, and the total blood sugar was estimated (13) at different time intervals (0, ½, 1, 2, 4, 8, 12 hr). These results were compared with those of the vehicle-treated controls and statistically analyzed (14; table 2).

A total of six healthy adult male langurs of different age groups with large canines, a well developed pinkish oedematous band, and the sexual skin on the rump were used as experimental non-human primate models. The animals were fed with wheat chapaty (unleavened bread), banana, onion, carrot, potatoes, and soaked Bengal grams and were provided with water *ad libitum*. Continuous veterinary supervision was maintained.

Polypeptide-p-$ZnCl_2$ (0.5 unit/kg in saline) was administered subcutaneously. Fasting blood sugar samples of each of the animals were taken before any dose of drug was given. Blood samples were taken at different time intervals, as shown in table 3. Food was given after 4 hourly blood samples were taken. An equal number of male langurs were kept fasting and injected with saline (0.9% NaCl in water); their blood sugar samples were taken according to the schedule in table 3.

CLINICAL TRIALS.—A total of nineteen patients (15 males and 4 females) suffering from primary idiopathic (15) diabetes mellitus (15–50 hr age group) for a period of three months to eight years were selected for clinical trials. Out of the nineteen patients selected 11 cases were of juvenile diabetes and 8 were of maturity onset diabetes. Diabetic patients suffering from ketoacidosis, cerebrovascular accidents, acute myocardial infarction and renal failure were excluded from this study.

All patients were admitted to medical wards of S.M.S. Hospital, Jaipur, 4–5 days prior to the commencement of the study. Long-lasting insulin was withdrawn from patients 72 hr

Table 2. Effect of polypeptide-p-$ZnCl_2$ (0.5 unit/kg)* on the blood sugar levels of fasting *Meriones hurrianae* Jerdon (gerbils) at different time intervals. (Blood sugar mg/100 ml)

Group No.	Treatment	Body wt. (g)	Fasting (12 hr)	½ hr	1 hr	2 hr	4 hr	8 hr	12 hr
1	Vehicle treated controls (35)**	67±3	95±5	93±3[a]	86±9[a]	90±11[a]	89±3[a]	87±5[a]	84±7[a]
	Sugar fall (%)			2.1±0.5	9.5±1.7	5.3±0.7	6.3±0.5	8.4±0.5	11.6±1.2
2	Polypeptide-p-$ZnCl_2$ (70)**	63±7	92±3[a]	71±7[a,c]	60±5[b,c]	36±7[b,c]	44±8[b,c]	47±8[b,c]	57±7[b,c]
	Sugar fall (%)			22.8±2.0	34.8±3.1	60.9±9.3	52.2±1.8	48.9±2.5	38.1±3.7

*1.3 mg/ml = 40 units.

**Figures in parentheses represents the number of gerbils examined (5 animals were used at each time interval in saline vehicle & 10 animals per time interval with polypeptide-p-$ZnCl_2$ treatment).

[a]Significant at 1% level compared with vehicle treated controls.

[b]Highly significant compared with vehicle-treated controls.

[c]Non-significant compared with vehicle treated controls.

[a]Non-significant compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.

[b]Significant at 1% level compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.

[c]Highly significant compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals. All figures are ± S.E.M.

[a]Non-significant compared with fasting sugar level of vehicle treated controls.

TABLE 3. Effect of polypeptide-p-$ZnCl_2$ (0.5 units/kg)* on the blood sugar level of fasting *Presbytis entellus entellus* Dufresne (Langurs). (Blood sugar mg/100 ml)

Group No.	Treatment	Body wt. (Kg)	Fasting (12 hr)	½ hr	2 hr	4 hr	20 hr	72 hr
1	Vehicle treated controls (3)**	12±3	62±3	62±5	64±7	58±5[a]	63±5	67±3
	Sugar fall (%)			NIL	NIL	6.5±3	NIL	NIL
2	Polypeptide-p-$ZnCl_2$ (3)**	13±5	64±5[j]	53±5[j,a]	29±1.3[j,b]	20±1.1[j,c]	31±7[k,a]	51±5[k,a]
	Sugar fall (%)			17.2±1.9	54.7±2.7	68±1.8	51.6±3.2	20.3±2.6

*1.8 mg/ml=40 units.
**Figures in parentheses represent the number of langurs examined.
[a]Significant at 5% level compared with vehicle treated controls.
[b]Significant at 1% level compared with vehicle treated controls.
[c]Highly significant compared with vehicle treated controls.
[d]Non-significant compared with vehicle treated controls.
[e]Significant at 5% level compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.
[f]Significant at 1% level compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.
[g]Highly significant compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.
[h]Non-significant compared with fasting sugar level of polypeptide-p-$ZnCl_2$ treated animals.
[i]Non-significant compared with fasting sugar level of vehicle treated animals.
All figures are ± S.E.M.

TABLE 4. Effect of polypeptide-p on blood sugar level in patients with diabetes mellitus.

	No. of subjects	Fasting values 7 A.M. (mean mg%)	Diabetes duration (yrs)	Mean mg% fall in blood sugar level						
				½ hr	1 hr	1.5 hr	4 hr	6 hr	8 hr	12 hr
JUVENILE DIABETES										
Controls	6	305 ± 10.5	4–8	298.3[a] ±3.0	294.4[a] ±3.7	294.3[a] ±3.0	292.5[a] ±3.4	291.6[a] ±3.9	291.9[a] ±4.2	293.1[a] ±4.6
Polypeptide-p	5	304 ± 13.9	4–8	253.4[a-c] ±27.4	210.7[a-c] ±37.4	187.6[a-c] ±41.6	168.7[a-c] ±46.8	176.0[a-c] ±42.9	172.4[a-c] ±40.7	208.6[a-c] ±38.6
MATURITY ONSET DIABETES										
Controls	2	145 ± 3.0	0.3–3.0	140.7[a] ±2.0	140.4[a] ±1.6	139.1[a] ±2.3	138.4[a] ±2.9	138.2[a] ±2.6	137.9[a] ±2.3	138.6[a] ±2.6
Polypeptide-p	6	140.9 ± 15.6	0.3–3.0	119.5[a-d] ±22.1	111.7[a-d] ±12.0	95.5[b-d] ±19.2	100.7[b-d] ±15.8	101.8[b-d] ±15.8	93.8[b-d] ±19.5	105.0[b-d] ±16.9

Juvenile Diabetes

[a] = Significant at 5% level compared with control.
[b] = Significant at 1% level compared with control.
[c] = Non-significant compared with control.
[d] = Non-significant compared with fasting sugar level of juvenile diabetic controls.
[e] = Non-significant compared with fasting sugar level of juvenile diabetes treated with polypeptide-p.
[f] = Significant at 5% level compared with fasting sugar level of juvenile diabetes treated with polypeptide-p.
All figures are ± S.E.M.

Maturity Onset Diabetes

[a] = Significant at 5% level compared with control.
[b] = Non-significant compared with control.
[c] = Non-significant compared with fasting sugar level of maturity onset diabetic controls.
[d] = Non-significant compared with fasting sugar level of maturity onset diabetes treated with polypeptide-p.

prior to the test, and plain insulin was withdrawn 12-18 hr before the test. Oral hypoglycemics were withdrawn 48 hr preceding the study. A blood sugar sample after the overnight fast was taken at 7 a.m. Polypeptide-p preparation in saline solution was administered subcutaneously in a dose depending on the severity of diabetes mellitus (less than 180 mg/100 ml blood sugar level, 10 units; 180–250 mg/100 ml blood sugar level, 20 units; 250 mg/100 ml of blood sugar or above, 30 units).

After administration of the polypeptide-p preparation, the first three samples were taken at half-hour intervals to record the onset of the hypoglycemic effect. Subsequent samples were taken at different time intervals, as shown in table 4, to show the peak effect and duration of the action of this polypeptide. The blood samples were withdrawn from the medial cubital vein. The subjects were kept fasting during the study; only plain lemon water was given, if desired by the patients. Supervision was maintained for administration of glucose upon development of hypoglycemic symptoms. Blood sugar determinations were performed by the method of Nelson-Somogyi (10).

The control group consisted of eight of the original nineteen patients with diabetes mellitus. Control blood samples were withdrawn at the same time intervals without the polypeptide p being administered (table 4). Polypeptide-p-$ZnCl_2$ was administered s.c. to three juvenile patients. These patients required smaller doses of this drug than on bovine insulin.

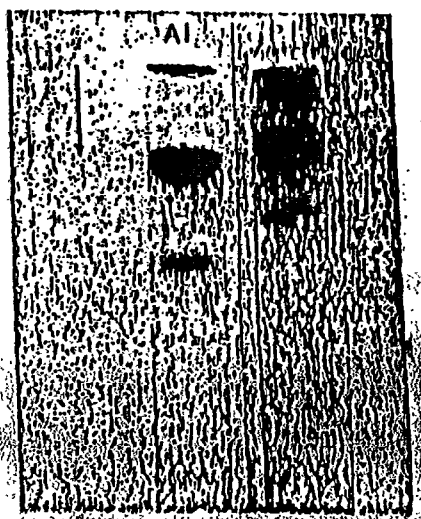

FIG. 1. Polyacrylamide gel electrophoresis pattern of bovine insulin (A1) and plant protein (polypeptide-p; P1) of *M. charantia*.

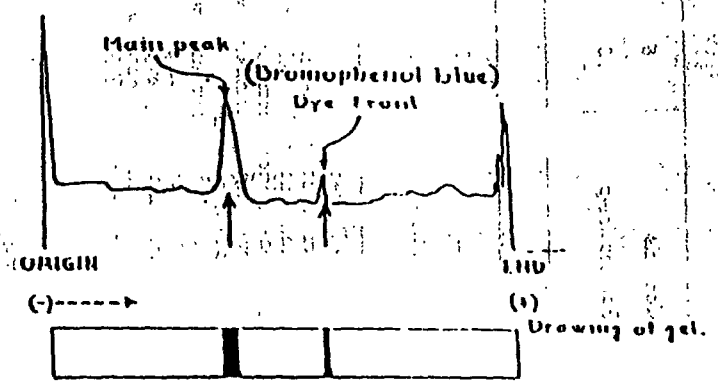

FIG. 2. Scanning of the polyacrylamide gel after electrophoresis on chromoscan (III of the main peak 0.41) of plant protein (polypeptide-p) of *M. charantia*.

RESULTS

A single electrophoretic band of dialyzed and crystallized substance (Rf 0.41) was observed which, however, did not coincide (Rf 0.47) with that of bovine insulin (fig. 1). On scanning, a single main peak (Rf 0.41) of pure polypeptide-p was observed (fig. 2).

Two-dimensional tlc and the amino acid analysis (automatic amino acid analyzer) of the polypeptide-p hydrolyzate showed 17 amino acids with a total of 100 residues and a minimum molecular weight of approximately 11000 (table 1). Methionine was the extra amino acid observed in the unknown samples when compared with that of the known bovine insulin. Bio-immunoassays of this polypeptide were found to be negative against bovine insulin.

The pharmacological study revealed that the polypeptide-p-$ZnCl_2$ was long acting in gerbils and langurs and showed a significant blood-sugar-lowering effect (table 2, 3).

Clinical trials showed a hypoglycemic effect of polypeptide-p in juvenile and maturity-onset diabetic patients (table 4). The peak effect in the juvenile diabetic may be between 4–8 hr as compared with 2 hr for crystalline bovine insulin. The peak response in maturity-onset diabetics is not as readily determined as in juvenile diabetics (table 4).

No complaints of any side effects followed administration of polypeptide-p-$ZnCl_2$ to the three juvenile patients. One juvenile patient who expressed frequent heaviness of the head, a swollen face, pain in the stomach, and recurrent episodes of hypoglycemia when kept on crystalline bovine insulin was free of these side effects when maintained continuously on polypeptide-p-$ZnCl_2$ for a period of five months. Immunoassays did not show any cross reaction when tested with bovine insulin.

DISCUSSION

Considering some of the resemblances of polypeptide-p with those of bovine insulin (i.e., extraction procedure, crystallization process, hypoglycemic activity, preparation of polypeptide-p-$ZnCl_2$ (6, 7) and potency), the crystalline isolate has been named p-insulin. However, due to certain differences (i.e. one extra amino acid methionine and negative immunoassays against bovine insulin) the question of a final name for the polypeptide remains open. No apparent side effects were observed when the p-insulin was screened in diabetic patients. Thus, considering its relative hypoglycemic potency and lack of antigenicity responses, p-insulin merits additional testing.

Bovine insulin so far is the only remedy against diabetes mellitus. With these new data, a new horizon in the treatment of diabetes mellitus may have been opened. Since the active principle is from a plant source, it is less likely to be antigenic. More clinical trials of action, antigenecity, and various effects of intermediary metabolism in human beings are in progress and shall be reported later.

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